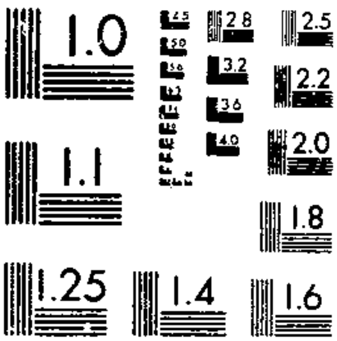
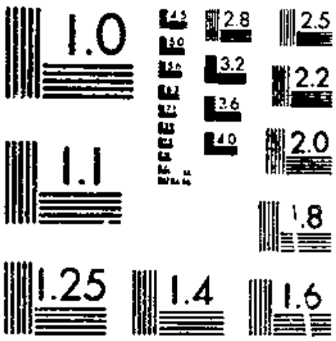
TB 977 (1949) USDA TECHNICAL BULLETINS UPDATA
STUDIES OF THE DIAPAUSE IN THE PINK BOLLWORM IN PUERTO RICO
FIFE, L.C. 1 OF 1

START



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

Technical Bulletin No. 977 • *January 1949*

UNITED STATES
DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

Studies of the Diapause in the Pink Bollworm in Puerto Rico[1]

L. Courtney Fife

Entomologist, Division of Cotton Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, in Cooperation With the Puerto Rico Agricultural Experiment Station

CONTENTS

INTRODUCTION

The diapause, or resting stage, of the pink bollworm (*Pectinophora gossypiella* (Saund.)) has been studied under widely different environmental conditions, but there is still disagreement as to the factors which initiate it and limit its duration. For 3 years behavior of larvae of this insect in Puerto Rico was studied in relation to definite changes in the environmental complex so that the factors responsible for the development and duration of the diapause in the field might be determined. Puerto Rico is an ideal place for the study of diapause phenomena, since low temperature, the apparent predisposing cause in many insects, is not encountered. In the laboratory controlled

[1] Received for publication September 7, 1939.

811498—49——1

experiments were conducted in which environmental changes were introduced in order that the interpretation of the effect of the environmental complex on the larval stage might be facilitated.

LITERATURE ON THE DIAPAUSE IN INSECTS

Several theories on diapause have been advanced, but none satisfactorily explains its causes or the factors limiting its duration. Pictet (*33–37*)[2] has advanced a theory that the diapause in insects is due to a hereditary rhythm more or less independent of external factors and resulting from a process of natural selection. Baumberger (*5*) and Roubaud (*41*) conclude that dormancy is brought about by self-intoxication of the organism due to an accumulation of excretory products in the tissues. Cousin (*19, p. 312*), as a result of experiments with *Lucilia sericata* (Meig.), maintains that the diapauses are due entirely to unfavorable environmental conditions, that they continue only so long as these unfavorable conditions exist, and that the phenomenon is not a necessary or obligatory biological process in the life of the insect. These conclusions were in part confirmed by the results of Strelnikov (*49*), since he concluded that under natural conditions the larval diapause in *Loxostege sticticalis* (L.) is brought about by insufficiently moist food, by food with an increased nutritive value, or by low temperature.

Uvarov (*53, p. 62*), Babcock (*2*), and Steinberg and Kamensky (*47*) attribute the diapause to a variety of causes, directly or indirectly due to external factors of the environment and complicated by manifestations of an acquired rhythm of development. Experiments by Spooner (*43*) and Townsend (*51*) on the codling moth have suggested enzymes as the cause of dormancy. Bodine (*12*) has advanced a theory that the embryonic diapause in *Melanoplus differentialis* (Thos.) is due to a hypothetical "diapause factor" which increases in amount and inhibits growth, but is gradually destroyed or itself inhibited during exposure to low temperature, thus liberating the inherent developmental factors. Ditman, Weiland, and Guill (*20*), experimenting with the corn earworm (*Heliothis armigera* (Hbn.)) concluded that the pupal diapause is caused by low temperature during the larval period and that neither the water content of the pupae nor the age of the corn on which the larvae were fed appeared to exert any influence on the tendency of the pupae to enter the diapause.

The most universally recognized difference between hibernating and nonhibernating insects is perhaps the difference in water content. Investigations by Bodine (*10, 11*), Breitenbecher (*15*), Fink (*24*), Hodson (*26, p. 290*), and Payne (*31, 32*) indicate that the period of hibernation is characterized by a reduction in the water content of the insect's body, whereas the amount increases after hibernation. Babcock (*1, 2*), Bodine (*10, 11*), Breitenbecher (*15*), Douglass (*21, 22*), Fink (*24*), Hodson (*26*), Rice (*38*), Townsend (*51*), and others have shown that the presence of moisture, usually contact moisture, is essential for the resumption of normal activity of various species of in-

[2] Italic numbers in parentheses refer to Literature Cited, p. 23.

sects after the diapause. In general, hibernation becomes longer than normal and is accompanied by a higher mortality in the absence of moisture. These facts suggest that an increase in the water content may be regarded as an essential process interrupting this resting condition. Breitenbecher (*15*), Bodine (*10*), and Buxton (*16*) have demonstrated that some species can even obtain this necessary moisture from saturated air or from moist soil.

Many investigators have shown that the diapause in a number of species is not caused by a reduced temperature, since many species enter a dormant condition in tropical areas or during the summer in temperate regions when the temperature is still favorable for development. Baumberger (*6*) concludes that organisms with a periodic diapause prepare for it at a certain time regardless of the temperature that obtains at that time. Chapman (*18, p. 122*) and Bodenheimer (*9 p. 143*), however, are of the opinion that almost any physical reaction of an organism may be shown to be due in part to environment and in part to heredity.

These theories as to the causes and termination of diapauses in insects have also been summarized by Boyce (*13*), Chapman (*18*), Cousin (*19*), Shelford (*42, p. 150*), Uvarov (*53*), and Steinberg and Kamensky (*47*).

With respect to the pink bollworm, Williams (*55*) in Egypt concluded that the factors determining the resting stage appear to be a combination of food, temperature, moisture, and rhythm. Based on a limited number of observations, Taylor (*50*), in Uganda, considered that the resting stage is induced by aestival conditions. He concluded that the resting stage is caused by any factor or factors that reduce the water content of the larvae, i. e., high temperature, low humidity, excessive evaporation, dry food, or lack of rainfall. He states that—

rainfall of 40'' to 60'' distributed through most months of the year, a relatively high mean daily humidity (exceeding 50%), and a mean daily temperature in the neighbourhood of 70° F. with a maximum rarely above 90° F and a minimum of about 50° F, together induce short cycle development without a diapause.

Ballard (*3*) noted that in India during the hot months the larval life was longer than later in the year, thereby suggesting that excessively high temperatures decrease the rate of development of the larvae. Wolcott and Sein (*56*), in Puerto Rico, found that the diapause develops during the months of January to April—the dry period on the south coast—while no resting larvae were found on the north coast during the months of September and October, the rainy season, or at the end of the harvest.

Squire (*44*), working in the Lesser Antilles, believes that the resting stage has little to do with weather or season but is in some way connected with the age of the crop. He concludes that the diapause was induced by dry and rich food toward the end of the crop irrespective of the time of year at which this takes place. He also found that larvae that develop from flowers or green bolls do not rest.

Bedford (*7*), in the Sudan, also concluded that the period during which the pink bollworm started to produce resting larvae varied according to the date of planting and the age of the crop. He believed that the factors directly responsible for the diapause were not entirely climatic or due to lack of food, but were influenced by chemical changes in the composition of the food as the plant reached a state of maturity. Storey (*48*), in Egypt, also found a rapid increase in long-cycle larvae (in the diapause) at the end of the season. However, Johnston (*28, p. 20*), in the Gezira of the Sudan, found that most of the larvae remaining after the destruction of the crop were of the short-cycle type. Bredo (*14*) states that in the Belgian Congo, as in Tanganyika, no resting larvae were found among the seeds at the end of the cotton season. Willcocks (*54, p. 136*), in Egypt, is of the opinion that the diapause is a condition of aestivation, an adaptation to a rainless period when its food plants are not fruiting. Gough (*25*), also in Egypt, states that it appears probable that hibernation of full-fed larvae is induced by the temperature of their surroundings at the time of their reaching maturity.

In the Laguna district of Mexico, Loftin, McKinney, and Hanson (*30*) regard the resting stage as a combination of aestivation and hibernation, since it begins in August while food is still abundant and the temperature is high. In November and December, when the temperature is lower and little food is available, the short-cycle larvae are exceptional. In the Big Bend of Texas, also, the diapause begins by the first part of August while temperature conditions are still favorable and food abundant. The Laguna District of Mexico and the southwestern part of Texas, where these studies were made, are both desert areas with extended drought periods, high temperatures, low humidity, and excessive evaporation. Cotton in both these regions is grown under irrigation.

CLIMATE OF PUERTO RICO

Studies in Puerto Rico were conducted at Isabela, Mayaguez, and Boqueron. As shown by the maximum and minimum records in table 1, rainfall in each of these localities is irregular and subect to great variation. At Isabela and Mayaguez the average annual rainfall is more than 50 inches and usually evenly distributed, except during December, January, February, and March—the dry period. The climate in these two areas favors the development of a mesophytic vegetation. The relative humidity is high, evaporation is not excessive, and plants have a considerable supply of water during most of the year.

At Boqueron, with an average rainfall of 28.95 inches, the climate favors the development of xerophytic vegetation owing to the frequent long periods of drought, low humidity, and warm, dry winds, which cause excessive evaporation. The drought is most pronounced during the 4 months of December to March, inclusive. Cotton is grown in this area without irrigation and almost always suffers from lack of moisture.

TABLE 1.—*Weather Bureau records of rainfall (inches) at the places where the diapause of the pink bollworm was studied in Puerto Rico*

Month	Mayaguez [1]		Isabela [1]		Boqueron (Enseñada)[2]	
	Average	Range	Average	Range	Average	Range
January	2.00	0.16– 4.67	3.40	0.0–11.65	0.85	0.0– 3.95
February	2.18	.0– 7.19	2.76	.22– 7.40	1.49	.0– 8.00
March	3.73	.12– 10.45	2.85	.49– 8.93	1.44	.0– 5.19
April	5.17	.55– 13.86	3.36	.15– 8.68	1.86	.0– 7.00
May	8.27	2.00– 17.45	6.40	1.95–17.06	3.39	.23– 9.07
June	8.97	3.32– 16.31	4.75	.85–16.27	2.16	.0–13.25
July	10.73	.80– 18.87	3.98	1.20–11.87	1.60	.06– 5.72
August	11.37	4.22– 19.02	5.20	.66–15.00	2.95	.0– 9.16
September	10.89	4.84– 24.40	5.67	1.00–16.80	4.34	.87–11.20
October	9.79	3.73– 21.51	5.33	1.08–14.50	3.98	1.44– 9.72
November	5.95	1.09– 16.69	6.94	2.01–20.20	3.69	.70– 9.64
December	2.60	T– 6.82	4.64	.60–12.74	1.20	.0– 3.85
Annual	81.70	58.43–112.28	55.28	35.90–93.50	28.95	11.34–53.07

[1] Period from 1899–1937.
[2] Period from 1902–1937.

In contrast to a Temperate Zone climate, that of Puerto Rico exhibits great stability of temperature. The mean daily temperature along the coast ranges throughout the year from 75° to 80° F., with a maximum rarely above 95° and a minimum of about 55°. The mean annual temperature averages about 77°. The usual range of temperature through the course of a single day is about 20° along the coast, and the fluctuation in the annual mean temperature from year to year does not exceed 3°.

SEASONS OF COTTON PRODUCTION

Puerto Rico has two cotton-growing sections with growing seasons at opposite periods of the year. On the north coast cotton is usually planted from January 1 to March 15, the picking season extends from July 1 to September 30, and the dead season occurs from October 1 to December 31. On the south coast, however, the cotton is planted from August 1 to September 30, the picking season occurs from January 1 to May 15, and the dead season extends from May 16 to July 31. In this southern area the planting season occurs during the period of greatest rainfall and the picking season during the dry period, whereas on the north coast this condition is reversed.

MOISTURE AS A FACTOR INDUCING THE DIAPAUSE

EFFECTS OF RAINFALL

To determine the abundance of resting larvae in the field, infested open bolls were placed on the soil surface in wooden cages 3 by 3 feet

FIGURE 1.—Pink bollworm cocoons spun between the bottom of a petri dish and a thin disk of absorbent cotton at Mayaguez, P. R.

by 10 inches in depth, covered on top with 16-mesh copper wire. Samples of the bolls were examined to determine the approximate number of larvae installed per cage. Thirty days after date of installation the bolls were examined and the number of living larvae recorded. When green bolls were used, the fourth instars were removed from the bolls and allowed to spin up in a petri dish between a thin layer of absorbent cotton and the bottom of the dish. As shown in figure 1, this technique permitted observations to be made without disturbing the larvae. The percentage of resting larvae was based on the proportion of live pupae to larvae in the dishes 30 days after date of installation.

To establish a reliable criterion for distinguishing larvae in the resting stage from short-cycle larvae the rate of pupation of 1,734 larvae removed from green cotton bolls was determined by the petri-dish method as described above. The results are shown graphically

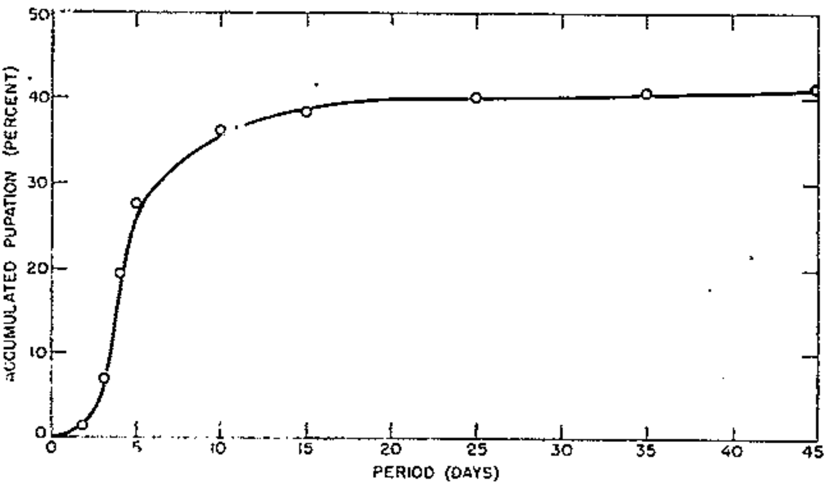


FIGURE 2.—Rate of pupation of fourth instars of the pink bollworm when removed from green cotton bolls, placed in petri dishes, and covered with cotton. Insectary at Mayaguez, P. R., 1937.

in figure 2. It will be noted that the rate of pupation during the first 10 days was very rapid, whereas after 20 days, as indicated by the nearly horizontal line, there was almost no pupation. Although no definite period can be established, it would seem logical to classify larvae as of the resting type if they do not pupate within 20 to 30 days after reaching maturity.

In Puerto Rico the abundance of pink bollworms in the diapause in fields of various ages at four localities were determined over a period of 2 years and these records have been correlated with rainfall in table 2 to show the relationship.

TABLE 2.—*Relation between rainfall occurring in the preceding 30, 45, and 60 days and the abundance of resting pink bollworm larvae in green and open cotton bolls, Puerto Rico, 1936–38*

Date of observation	Locality	Growth stage of bolls	Records	Resting larvae	Total rainfall during stated periods prior to date of observation		
					30 days	45 days	60 days
			Number	*Percent*	*Inches*	*Inches*	*Inches*
1936							
Jan. 8	Isabela	Open [1]	60	92	1. 46	7. 52	9. 58
Jan. 10	do	do	717	65	1. 46	[illegible]. 52	9. 58
June 15	do	do	271	1	16. 21	23. 33	23. 73
July 14	do	do	108	1	5. 60	12. 71	21. 81
20	do	do	452	2	4. 61	11. 45	19. 09
Aug. 11	do	do	270	1	7. 30	8. 64	12. 81
Do	do	do [2]	270	4	7. 30	8. 64	12. 81
Sept. 4	do	do	553	0	9. 47	15. 35	16. 32
Do	do	do [2]	553	3	9. 47	15. 35	16. 32
June 23	Boqueron	do	471	5	3. 30	10. 60	16. 10
Aug. 1	do	do [2]	374	25	1. 10	1. 10	4. 40
1937							
Aug. 19	Isabela	Green [1]	472	0	7. 41	8. 93	10. 86
Sept. 15	do	do [1]	200	1	2. 25	7. 14	9. 62
30	do	do [1]	146	8	4. 46	5. 99	10. 88
Nov. 9	do	do [1]	114	26	2. 19	3. 69	6. 23
Mar. 26	Boqueron	Open [1]	151	89	0	0	. 60
Apr. 17	do	do	389	100	1. 30	1. 30	1. 30
Oct. 15	Mayaguez	Green [1]	128	0	10. 46	13. 32	22. 60
Nov. 23	do	do [1]	200	4	8. 49	12. 62	22. 17
10	Hatillo	do [1]	71	37	4. 23	4. 85	5. 64
1938							
Jan. 10	Isabela	do [1]	297	46	4. 83	5. 26	9. 07
Do	Mayaguez	do [1]	305	70	. 45	. 76	3. 86

[1] Larvae were removed from the bolls and allowed to spin cocoons between the bottom surface of petri dishes and thin disks of absorbent cotton and maintained thereafter under insectary conditions.
[2] Bolls were placed under a shelter to protect them from the sun and rain.

It will be noted that larvae in the diapause were abundant only following periods of 2.5 inches or less of rainfall 30 days prior to the date of observation, or 5.5 inches or less of rainfall 45 days prior thereto. A few long-cycle or resting larvae, occurred regardless of the amount of rainfall. This relationship was most clearly shown at Isabela. As shown in figure 3, resting larvae were abundant during January, February, March, and April, 1936, a period of severe drought. Very few resting larvae occurred from June to December, a period of heavy rainfall. During another period of drought, November 1937 to January 1938, the pink bollworms in diapause were again abundant. As shown in table 2, resting larvae occurred in abundance at Mayaguez only during the drought period. At Boqueron cotton plants usually suffer greatly from lack of available moisture during the major part of each growing season, and as a result pink bollworms in the diapause are usually abundant at any period of the year when cotton is being grown.

Taylor (*50*), in Uganda, found that larvae of the pink bollworm do not normally rest where the rainfall of 40 to 60 inches is well distributed throughout the year, whereas at Isabela and Mayaguez, P. R., which have about the same amount of rainfall but of less even distribution, the diapause is common during the dry season.

These studies indicate that the bollworms in diapause occur in abundance in Puerto Rico during periods of drought regardless of the age of the crop or the time of the year at which this drought takes place. Also, a few resting larvae occur regardless of the amount of

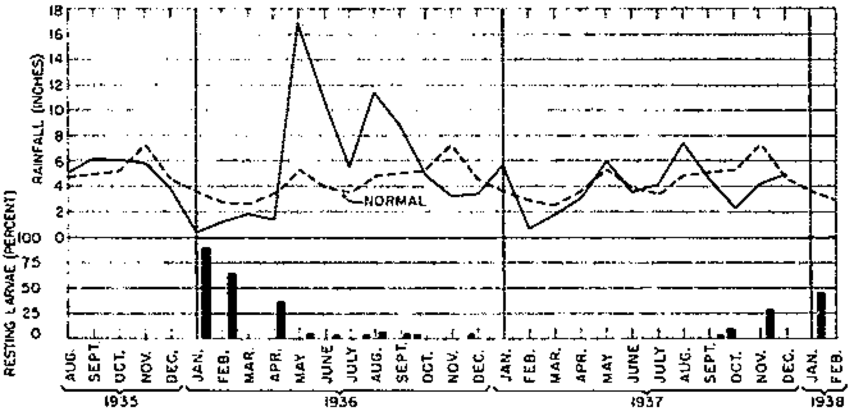


FIGURE 3.—Relation between rainfall and the abundance of resting larvae of the pink bollworm from August 1935 to February 1938 at Isabela, P. R. The heavy vertical bars indicate the occurrence and abundance of resting larvae, the broken line the normal rainfall, and the solid line the rainfall of the years indicated.

rainfall—an indication of the existence of an inherited cycle that cannot be broken within several generations.

Reaction of Larvae to Artificial Drought Conditions in the Laboratory

Field studies showed that a high percentage of the larvae develop into a resting stage on plants suffering from a deficiency of water. To confirm these field observations, a study was made of the reaction of pink bollworm larvae developing in a greenhouse on plants receiving an abundant supply of water and on plants suffering from water deficiency.

METHODS OF STUDY

On July 7, 1937, cotton was planted in 176 large cans filled with sandy loam soil to which one-fourth pound of fertilizer per can had been added. Five holes had been cut in the bottom of each can to permit drainage and approximately 2 inches of gravel had been placed in the bottom of each can, and the cans then set on 2- by 2-inch rails on a platform in the greenhouse. After many bolls had been set, 85 days after planting, the amount of water supplied to 132 plants was cut down so as to cause severe wilting and shedding of the leaves and fruiting forms. The plants of this series, which averaged 4 feet in height, are illustrated in figure 4. The other plants were watered daily. These well-watered plants were continuously turgid, vigorous, and healthy, bearing many fruiting forms per plant.

Blooms on all plants were tagged daily so that the age of the bolls would be known. From October 20 to 27 several first-instar larvae were placed on all tagged bolls regardless of age. On November 12 all the larvae were removed from the bolls to the insectary and classi-

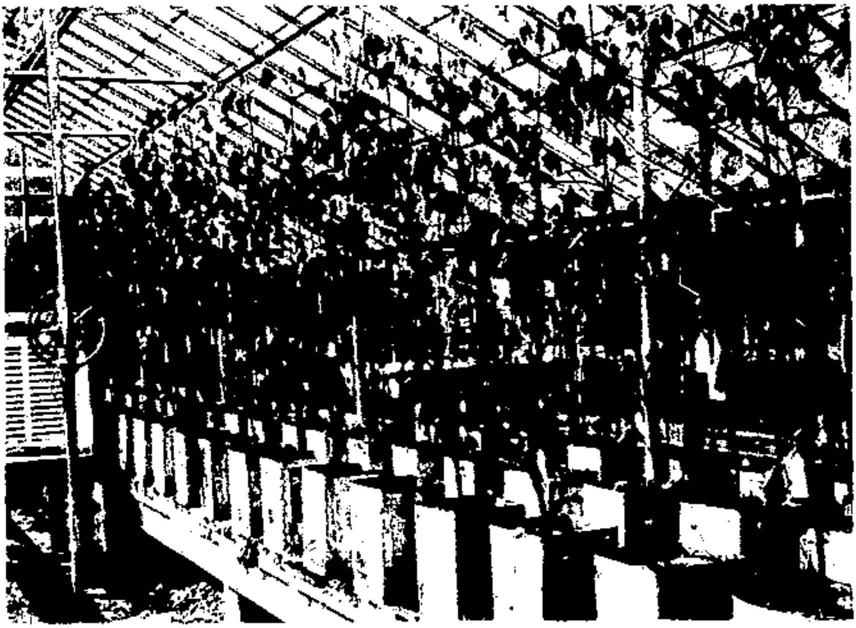


FIGURE 4.—Cotton plants growing in 5-gallon oil cans in the greenhouse at Mayaguez, P. R. Their water supply was so deficient as to cause severe wilting and heavy shedding of the leaves and fruiting forms.

fied as to the conditions under which they had developed. The larvae spun up between the bottom surface of Petri dishes and thin disks of absorbent cotton, as shown in figure 1. By the use of this method observations could be made without disturbing the larvae.

Temperature and relative humidity were recorded in the insectary and greenhouse by the use of hygrothermographs. The greenhouse was covered with transparent glass while the sides were enclosed with fine-mesh wire. The mean daily temperature in the greenhouse was 82.4° F. (4.1° higher than in the insectary), the maximum temperature recorded in the greenhouse was 101° (11° higher than the maximum temperature recorded in the insectary over the same period), and the mean daily relative humidity in the greenhouse was 83.3 percent (3.7 percent higher than in the insectary). These conditions, however, are probably not at all similar to those encountered in the field.

RELATION BETWEEN THE AGE OF COTTON BOLLS AND MOISTURE CONTENT OF THE SEED AND LINT

The moisture content of the seed and lint, or locks, of green and open bolls of various ages was determined on October 23, 1937, and again on November 12, by drying the locks in an oven at 215° F. for 48 hours. As shown in table 3, in all cases except one the moisture content of the seed and lint in the normally watered series was higher than in the deficiently watered series for each age group, this difference becoming greater as the age of the boll increased. When the eight paired observations on October 23 were compared, the mean difference of 4.8 percent between the two series was significant. On November 12 these differences were much greater, but only two paired observations were made.

TABLE 3.—*Relation between the age of cotton bolls grown on deficiently watered and well watered plants in the greenhouse and the moisture content of the seed and lint on Oct. 23 and Nov. 12, Mayaguez, P. R., 1937*

Stage	Age of bolls	Plants normally watered, Oct. 23: Samples [1]	Plants normally watered, Oct. 23: Average moisture content	Plants normally watered, Nov. 12: Samples [1]	Plants normally watered, Nov. 12: Average moisture content	Plants deficiently watered, Oct. 23: Samples [1]	Plants deficiently watered, Oct. 23: Average moisture content	Plants deficiently watered, Nov. 12: Samples [1]	Plants deficiently watered, Nov. 12: Average moisture content
	Days	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Green bolls	27	5	79.9			2	77.7		
	31	3	74.7			3	75.8		
	35	4	70.2	3	76.6	4	69.6		
	40	3	64.6			4	61.4		
	41	1	63.8			2	56.9		
	42	1	60.6			2	52.7		
	43	1	63.4			1	53.4		
	44	1	61.8	1	63.1	1	53.0	1	46.4
	45					1	48.6		
	47			1	57.9			1	39.4
	51							1	36.9
	52			2	53.3				
Open bolls	44			1	36.7				
	47			1	33.8				
	51							1	18.3
	52			1	28.4				
	56							1	26.0

[1] Each sample contained from 3 to 9 locks.

In figure 5 it will be noted that the moisture content of the seed and lint was inversely proportional to the age of the boll, regardless of whether the plants suffered from water deficiency. The coefficient of correlation between these two factors on October 23 in the normally watered series was −0.986. When the regression was calculated, it showed that, within the age limits studied, an average decrease of 11.3 percent in the moisture content of the seed and lint is to be expected for every 10 days' increase in the age of green bolls. Squire (*46*), in St. Vincent, obtained similar results, since he found that the moisture content of cotton seed decreased from 78.5 to about 14 percent within 8 days after the bolls began to open.

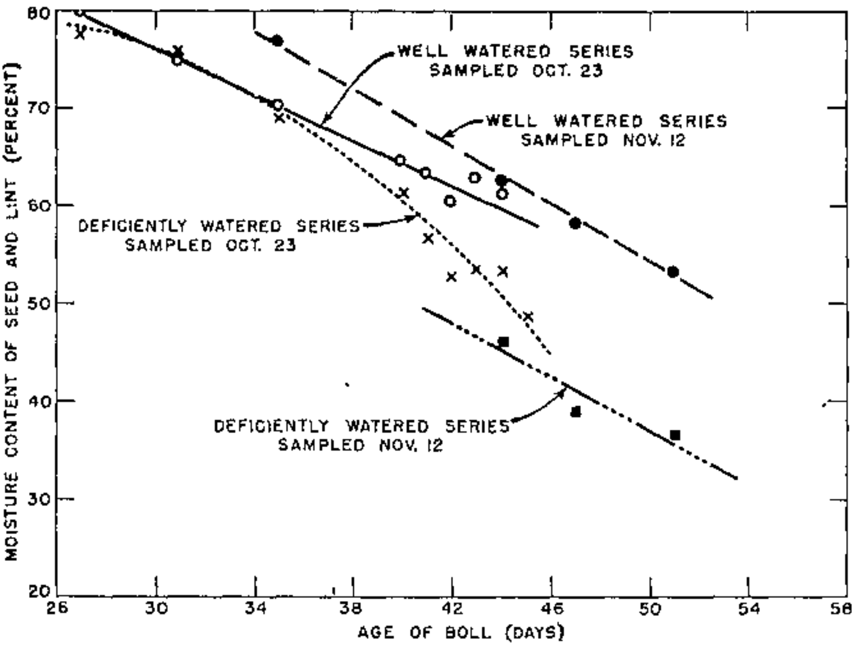


FIGURE 5.—Relation between the age of green cotton bolls on plants well watered and deficiently watered in a greenhouse and the moisture content of seed and lint. Mayaguez, P. R.

RELATION BETWEEN THE MOISTURE CONTENT OF THE SEED AND LINT AND OCCURRENCE OF THE DIAPAUSE

The relation between the moisture content of the seed and lint and abundance of larvae in the diapause was also determined. As shown in table 4, the percentage of resting larvae from bolls on deficiently watered plants was always higher than the percentage of long-cycle or resting larvae from bolls grown on the normally watered plants, the averages being 85.2 and 56.7 percent, respectively. The difference of 28.5 percent proved to be highly significant. The moisture content of the seed and lint of bolls grown on the deficiently watered plants was always lower than the moisture content of the seed and lint of bolls grown on the normally watered plants for each age group. By combining all the records in table 4 it was found that the moisture content of the seed and lint was inversely proportional to the percentage of resting larvae (fig. 6). However, the coefficient of correlation (−0.397) was not significant.

TABLE 4.—*Relation between the percentage of resting pink bollworm larvae, moisture content of the seed and lint, and age of cotton bolls grown on normally watered and deficiently watered plants in the greenhouse, Mayaguez, P. R.*

Age of bolls (days)		Plants normally watered			Plants deficiently watered		
		Records	Resting larvae	Moisture content of seed and lint	Records	Resting larvae	Moisture content of seed and lint
		Number	*Percent*	*Percent*	*Number*	*Percent*	*Percent*
Green bolls	(1)	106	41.5	73.4			
	35	167	50.3	76.6	68	[1] 79.4	[1] 62.9
	44	144	49.3	63.1	44	97.7	46.4
	47	146	71.2	57.9	66	90.9	39.4
	51	54	72.2	53.3	47	83.0	36.9
Open bolls	44	108	42.6	36.7			
	47	157	65.0	33.8			
	51	226	59.7	28.4	103	80.6	18.3
	56	55	61.8		37	86.5	26.0
Total or average		1,163	56.7	52.9	365	85.2	38.3

[1] Age of bolls not definitely known but estimated to be about 35 days.

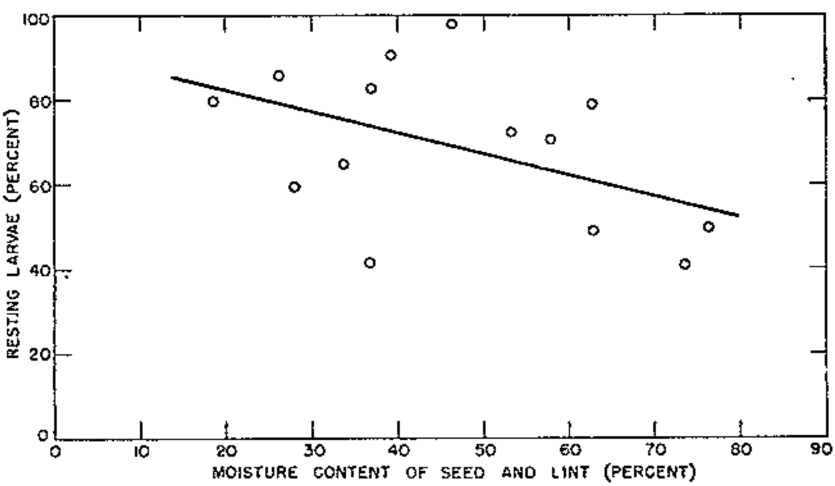


FIGURE 6.—Relation between the moisture content of the seed and lint from cotton bolls and the percentage of resting larvae of the pink bollworm. Greenhouse record, Mayaguez, P. R., 1937.

Even though the normally watered series of plants received daily applications of water, it is quite possible that they suffered to a certain extent from lack of moisture owing to the fact that they were growing in a rather limited space. This may help to explain why a rather high percentage of larvae from this series entered the diapause.

Squire (*45*, *46*), in St. Vincent of the West Indies, found a correlation of −0.397 between the moisture content of cotton seed and percentage of larvae in the diapause, the results being based on 647 larvae collected in young and open bolls in the field. The average moisture content of the insect's food during the larval period was determined by measuring the moisture content of the seed at various stages of maturity, but usually larvae have reached the second and often the third stadium before entering the seed to complete larval development. Squire also found that the oil content of the seed increased with the age of the seed, from 9.74 percent at 34 days to 24.6 percent at 44 days, and to 27.32 percent at 56 days. Also, the ether extract (fat) of resting larvae was about 15 percent higher than that of nonresting larvae, which might be due to feeding by the larvae on the older seed of a higher oil content.

Nevertheless these experiments, in conjunction with the field studies, indicate that dry food caused by drought conditions decreases the rate of metabolism of the larvae and consequently arrests development.

Relation Between Age of Bolls, Occurrence of the Diapause, and Moisture

To supplement the foregoing experiment the percentage of resting larvae and the moisture content of the seed and lint in young (succulent) and old (hard) green cotton bolls collected at Isabela were determined. The highest percentage of resting larvae (26.3) was found in the older bolls that contained the lowest percentage of moisture content (71.9), as compared with 11.2 percent of resting larvae in the younger bolls that had 81.6 percent of water, these results being based on 114 and 143 records, respectively. The difference of 15.1 percent of resting larvae may be regarded as highly significant, as the χ-square test indicates an association between the factors.

Two similar comparisons were also made at Hatillo but there was no difference in the percentage of resting larvae (36.7, 36.6), although the moisture content of the two samples of green bolls ranged from 82.3 to 61.5 percent. These results were based, however, on only 30 and 70 larvae, respectively.

Since bolls in various stages of maturity vary greatly in their moisture content, it was deemed important to determine the percentage of long-cycle larvae that develop in bolls of different ages. Bolls were collected in the same field during periods both of drought and of heavy rainfall and divided into four groups as follows: Young green bolls (succulent), old green bolls (hard), young dry bolls (partly broken loose when very young but not dropped from the plant), and mature open bolls.

As shown in table 5, the highest percentage of resting larvae occurred in the older fruiting forms which contained the least amount of moisture. It will also be noted that insects in the diapause occurred in much greater abundance during the period of drought.

TABLE 5.—*Relation between the percentage of resting pink bollworm larvae in cotton bolls of different ages during periods of drought and heavy rainfall, Mayaguez, P. R., 1937–38*

HEAVY RAINFALL: OCTOBER, 10.32 INCHES; NOV. 1 TO 23, 7.33 INCHES

Date of observation	Type of fruiting form	Records	Resting larvae
		Number	*Percent*
Nov. 23, 1937	Young green bolls (succulent)		
	Old green bolls (hard)	200	3.5
	Young dry bolls [1]	30	3.3
	Mature open bolls	123	20.3

LIGHT RAINFALL: DECEMBER, 0.5 INCHES; JAN. 1 TO 20, 1.6 INCHES

Date of observation	Type of fruiting form	Records	Resting larvae
Jan. 20, 1938	Young green bolls (succulent)	95	66.3
	Old green bolls (hard)	412	75.7
	Young dry bolls [1]	195	79.5
	Mature open bolls	340	92.4

[1] Bolls were partly broken loose when very young but did not drop off the plant.

RELATION BETWEEN THE AGE OF COTTON AND OCCURRENCE OF THE DIAPAUSE

According to Squire (*44*),

> The diapause is induced by dry and/or rich food toward the end of the crop irrespective of the time of the year at which this takes place. The field conditions leading up to this position are (*a*) the increase in the ratio of ripe to green bolls and (*b*) a sufficiently high incidence of the pest to necessitate the infestation of ripe and ripening bolls.

As shown in table 6, the percentage of resting larvae was determined monthly in the same field from June to September, 1936, at Isabela. The total rainfall occurring in the preceding 30 and 45 days prior to date of observation was also recorded. The percentage of long-cycle larvae did not exceed 4 percent, yet the incidence of the pest during this season was one of the highest on record. On August 15 the green-boll infestation in eight fields at Isabela ranged from 23 to 100 percent, averaging 71.1 percent. These observations shown in table 6, were made in a field having the highest infestation. It will also be noted that there was an abundance of rainfall during the period, the least amount being 4.61 inches during the 30 days prior to the date of observation.

TABLE 6.—*The relation between rainfall for periods of 30 and 45 days prior to date of observation and the abundance of resting larvae of the pink bollworm in open cotton bolls from the same field at Isabela, P. R., 1936*

Date of observation	Records	Resting larvae	Total rainfall during stated periods prior to date of observation	
			30 days	45 days
	Number	*Percent*	*Inches*	*Inches*
June 15	271	1	16. 21	23. 33
July 14	108	1	5. 60	12. 71
July 20	452	2	4. 61	11. 45
Aug. 11	270	4	7. 30	8. 64
Sept. 4	553	3	9. 47	13. 50

To elucidate this relationship further, the abundance of larvae in the diapause was determined in three fields of cotton of various ages. These fields were planted at Mayaguez on June 20, July 20, and August 20, 1937. The percentages of resting larvae in green bolls collected at random were determined in each field on January 10, 1938.

TABLE 7.—*Relation between the age of cotton plants and percentage of resting pink bollworm larvae in green bolls, Mayaguez, P. R., Jan. 10, 1938*

Age of cotton (months)	Records	Resting larvae
	Number	*Percent*
4.5	305	69. 5
5.5	307	67. 4
6.5	339	54. 3

As shown in table 7, the highest percentage of resting larvae was not found in the field of the oldest cotton but in that of the youngest. Since the oldest cotton was putting on its second crop, the moisture content of these bolls was probably higher than that of bolls from the youngest cotton, and this might explain the differences in results. Obviously these data do not support Squire's theory regarding the diapause.

EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ACTING TOGETHER ON THE DEVELOPMENT OF THE DIAPAUSE

To determine the combined effect of temperature and relative humidity acting together in causing the development of the diapause, numerous nonresting fourth instars were removed from green bolls and placed in dessicators where constant relative humidities ranging from 0 to 100 percent were maintained at various high temperatures. As a check, larvae were held at room temperature and relative humidity.

In the first experiment an average temperature of 98° F. was maintained, ranging from 95° to 104°; in the second test an average temperature of 95° was maintained, with a range of 90° to 100°.

Humidities were obtained by the use of saturated salt solutions, concentrated sulfuric acid, and anhydrous calcium chloride. Concentrated sulfuric acid was used for 0 to 1 percent humidity, anhydrous calcium chloride for 1 to 5 percent, sodium chloride for 75 to 78 percent, potassium sulfate for 90 to 98 percent, and distilled water for approximately 100 percent.

The results were based on the proportion of live larvae and pupae 30 days after the beginning of the experiment in accordance with the criterion established in a previous test as described on page 7.

TABLE 8.—*Effect of temperature and relative humidity during a 30-day period on pupation of fourth instars of the pink bollworm removed from green cotton bolls, Insectary, Mayaguez, P. R., 1937*

Relative humidity (percent)	Experiment No. 1				Experiment No. 2			
	78° F., room temperature [1]		98° F.[2]		77° F., room temperature [1]		95° F.[3]	
	Records	Larvae not pupating	Records	Larvae not pupating	Records	Larvae not pupating	Records	Larvae not pupating
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Check [4]	146	8.2			114	26.3	-	-
0-1	153	13.7	57	54.4	44	11.4	90	45.6
1-5	140	16.4	66	47.0	143	33.6	96	57.3
75-78	129	17.1	69	29.0	-		-	-
90-98			78	0	130	17.7	87	1.1
100	145	0	76	1.3		-	-	-

[1] Range 69° to 90° F.
[2] Range 95° to 104° F.
[3] Range 90° to 100° F.
[4] Check held at room conditions, average relative humidity 83 percent, range 56 to 96 percent.

Table 8 shows that the highest percentage of larvae not pupating (resting larvae) were found in treatments maintained at 75 to 78 percent relative humidity, or lower, and at the higher temperatures. Very few resting larvae were found in treatments at 90 to 100 percent relative humidity regardless of the temperature, the percentage in all cases being less than that of the check.

As shown in figure 7, the results of the two experiments are in close agreement. At 0 to 1, 1 to 5, and 75 to 78 percent relative humidity the percentage of resting larvae was always significantly higher at the higher temperature. At 90 to 100 percent relative humidity very few long-cycle larvae occurred. The experiments at 0 to 1 and 1 to 5 percent relative humidity were considered almost identical and, as expected, the results were the same.

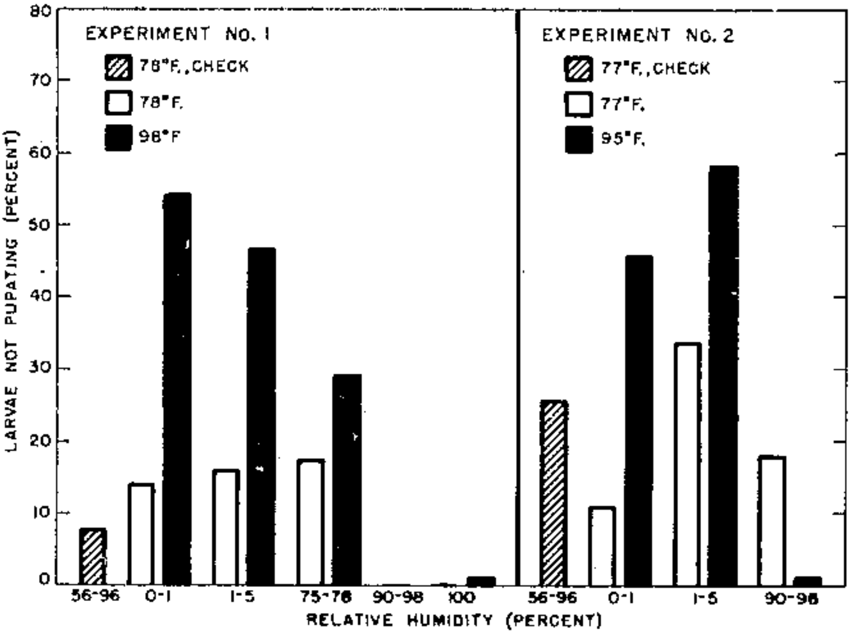


FIGURE 7.—Effect of temperature and relative humidity during a 30-day period on pupation of fourth instars of the pink bollworm removed from green cotton bolls. Insectary, Mayaguez, P. R., 1937.

Many investigators have shown that insects lose weight, presumably through loss of water, more rapidly under conditions of high temperature and low humidity than with low temperature or high humidity. The foregoing results would indicate that an increased percentage of nonpupating larvae (resting larvae) was found under conditions of high temperature and low humidity because of a greater loss in water by evaporation. This decreased the rate of metabolism and caused arrested development. These results are in agreement with the observations of Taylor (*50*) in Uganda.

MOISTURE CONTENT OF RESTING AND NONRESTING LARVAE AND ITS PROBABLE SIGNIFICANCE

The foregoing investigations indicate that the moisture content of the insect's food greatly influences its future development, i. e., whether the mature larvae will pupate immediately or enter the diapause. It has long been known that the moisture content of an insect's food greatly influences the water content of its tissues. It was, therefore, deemed important to measure the moisture content of resting and nonresting larvae. When larvae were dried in an oven at 215° F. for 48 hours it was found that the average moisture content of 25 nonresting larvae had been 70.8 percent and of over 200 resting larvae it had been 62.9 ± 0.46 percent, with a range in the latter case of from 60.8 to 65.4 percent. The difference of 7.9 percent was probably caused by the difference in the moisture content of their food. Squire (*46*) found that resting larvae often had a moisture content as much as 10 percent lower than that of normal larvae.

These results are of special interest when compared with the findings of other workers in connection with this subject. Robinson (*39*, *40*) found that a direct relationship exists between the water content of various species of insects and their food, species which live on food containing a low percentage of water having a small amount of water in their own tissues. He further showed that some species of insects that live under low moisture conditions have the remarkable capacity of adapting themselves to such conditions by binding on the surface of their colloids a large proportion of the available water and in this way protecting themselves against loss by evaporation.

Ditman, Weiland, and Guill (*20*), experimenting with the corn earworm (*Heliothis armigera* (Hbn.)), found that pupae in the diapause lose water less rapidly at low humidities than pupae not in the diapause.

These studies on the pink bollworm indicate a similar relationship between the moisture in the food and in the insect's tissues. Under tropical conditions changes in the environmental complex that affect the moisture content of the cotton boll are the most important factors inducing or hindering the development of the diapause. The moisture content of the food depends on the age of the boll and the amount of water available to the cotton plant.

TERMINATION OF THE DIAPAUSE

Many workers have shown that moisture, usually contact moisture, increases survival and expedites emergence of various insects from dormancy. With respect to the pink bollworm, Ballou (*4*, *p. 41*), in Egypt, found that when old infested bolls were kept dry the quiescent period was longer. Loftin, McKinney, and Hanson (*30*), in Mexico, found that dampening the seed and lint containing resting larvae hastened emergence. Williams (*55*) is of the opinion that pupation of larvae in the diapause, in Egypt, is largely controlled by temperature and humidity, and suggests the possibility that pupation may be controlled in the field by irrigation. Bishara (*8*, *p. 64*), also in Egypt, found that under continued cool and dry conditions the larvae rarely, if ever, pupate. Bedford (*7*) found that moths in the northern Sudan emerged from resting larvae chiefly during the period from the middle of July to the end of September, the rainy season. Kahn (*29*) also found that in the Punjab the peak of pupation and emergence occurred during the rainy season. Chapman and Cavitt (*17*), in experiments conducted under insectary conditions in Texas, found that the peak of moth emergence from hibernating larvae was reached during May, when the soil moisture was above 5 percent, and in July, when the soil moisture was below 5 percent, regardless of the type of soil. They also found that survival was increased in sandy and clay adobe soil with an increase of soil moisture up to a maximum of 17 percent, after which it decreased. Taylor (*50*), in Uganda, experimenting with nine resting larvae of the pink bollworm, found that a relative humidity of 100 percent terminated the diapause in all cases within 22 days. Squire (*44*), in the West Indies, says, "The addition of water to the tissues of resting larvae in most cases expedites emergence, but is not a *sine qua non* thereof and in a small percentage of cases fails to terminate the diapause." He also concluded (*46*) that the application of water to resting larvae reduces their fat content probably by facilitating hydrolysis, thus bringing them into line with normal larvae. Isler and Fenton (*27*), and Fenton and Owen (*23*) found that in Texas irrigation applied during the period of hibernation increased survival of the pink bollworm in cotton bolls on the soil surface.

Effects of Moisture on the Duration of the Diapause

An experiment was conducted at Boqueron to determine the effect of rainfall and temperature on the duration of the diapause in open cotton bolls on the soil surface. Heavily infested open cotton bolls were placed on the soil surface under wooden cages, 3 by 3 feet by 10 inches, which were covered on top with fine-mesh copper wire. Ten cages, each containing approximately 374 fourth instars in open bolls, were installed per cage. Five of these cages were placed under a shelter to protect them from the sun and rain and the other 5 were placed in the open. The bolls in 1 cage of each series were examined about every 35 days after date of installation.

Table 9.—*Survival of resting pink bollworm larvae in open cotton bolls on the soil surface in the open and under a shelter, Boqueron, P. R., 1936–37*

Date of examination	Period after date of installation	Live larvae recovered in—			
		Cages in the open		Cages under shelter	
	Days	*Number*	*Percent*	*Number*	*Percent*
Aug. 1	0	374	100.0	374	100.0
Aug. 31	30	7	1.9	94	25.1
Sept. 30	60	6	1.6	50	13.4
Nov. 13	104	0	0	22	5.9
Dec. 28	149	0	0	2	.5
Jan. 20	172	0	0	3	.8

Table 9 shows that in cages under the shelter the survival of resting larvae was much greater than among the larvae in exposed cages. In the sheltered series, 3, or 0.8 percent, of the larvae had not pupated 172 days after the date of installation, whereas in the open series no living larvae or pupae were found after 60 days. Since many weeds grew in the open cages, the bolls were shaded during most of the period, and the difference in the results was therefore attributed mostly to rainfall. During the first 60 days after the date of installation 5.10 inches of rain fell in this area.

In field observations the maximum duration of the diapause of larvae in open cotton bolls on the soil surface was 172 days at Isabela during a period of severe drought and 127 days at Boqueron. In the latter case, however, drought conditions were not so severe.

In another experiment conducted in the insectary 450 open cotton bolls, collected on March 16, were placed in each of 2 cages on April 17. An examination of a 200-boll sample showed that there were approximately 400 live fourth instars installed per cage. In 1 cage the bolls were kept continuously moist by submerging them frequently in water, while in the other cage the bolls were left dry. The 32 days between the date of collection and the date the experiment was initiated assured that all the larvae present were in the diapause.

TABLE 10.—*Comparative rate of emergence, by 20-day periods, of pink bollworm moths from resting larvae in 450 open cotton bolls in each cage when held under dry and under moist conditions in the insectary, Mayaguez, P. R., 1937*

Date	Period after date of installation	Accumulated emergence from—			
		Dry cotton bolls		Moist cotton bolls	
	Days	*Number*	*Percent*	*Number*	*Percent*
Apr. 17	0	0	0.0	0	0.0
May 7	20	0	0	9	10.3
May 27	40	0	0	69	79.3
June 16	60	11	2.6	87	100.0
July 6	80	90	21.5		
July 26	100	173	41.4		
Aug. 15	120	298	71.3		
Sept. 4	140	363	86.8		
Sept. 24	160	396	94.8		
Oct. 14	180	402	96.2		
Nov. 3	200	405	96.9		
Nov. 23	220	410	98.1		
Dec. 13	240	414	99.0		
Dec. 29	256	418	100.0		

The results of this experiment are summarized in table 10. Although a heavy mortality (approximately 78 percent) occurred in the moistened bolls, the addition of moisture greatly accelerated emergence. Fifty percent of the moths had emerged from the moistened bolls after approximately 32 days from the date of installation and all had emerged after 54 days. From the bolls kept dry 50 percent had not emerged until approximately 110 days had passed, and all had not emerged until after 256 days.

In another experiment resting larvae were allowed to spin up between the bottoms of petri dishes and disks of lint cotton (fig. 1). The dishes were then divided into 3 treatment lots as follows: (1) Cocoons kept moist continuously by daily applications of water, (2) those held with the atmosphere at 100 percent relative humidity, and (3) those in the dry check. The number of larvae used per treatment ranged from 62 to 99. Pupation was completed in 36, 54, and 135 days, respectively.

In a similar test where the cocoons of long-cycle larvae were moistened daily, weekly, and biweekly, pupation was completed in 83, 121, and 135 days, respectively. In a check that was left dry pupation was not completed until 147 days. The number of larvae used per treatment ranged from 161 to 184. The tissue paper which covered the cocoons in this experiment remained wet for about 8 hours after each application of water, but the water apparently failed to penetrate the cocoons in any of the foregoing treatments.

In other tests, where water had been forced into the cocoons by the use of a hypodermic needle, the larvae left the cocoons as soon as the water was applied.

These results show that moisture is the important factor in limiting the duration of the diapause under tropical conditions and that in the field, under conditions of heavy rainfall, pupation and emergence of a majority of the resting larvae would be completed within 2½ to 3 months. They also indicate the possibility of a practical use of moisture in reducing pink bollworm damage, since the occurrence of the diapause in the field, and therefore the carry-over, may be partly controlled by adjusting the planting date to the distribution of rainfall or by irrigation.

SUMMARY

The behavior of the larvae of the pink bollworm (*Pectinophora gossypiella* (Saund.)) has been studied in relation to definite changes in the environmental complex over a period of 3 years to determine the combinations of factors that initiate and terminate the diapause in the field. In the laboratory, controlled experiments were conducted in which environmental changes were introduced to aid in the interpretation of the effect of the environmental complex on the larval stage.

Puerto Rico offered distinct advantages for a study of this phase of insect life, as it has a tropical climate, and the factor of extreme cold as a predisposing cause of the diapause is excluded. Also, there is a portion of the island where cotton is grown during the dry season, and in another part of the island it is grown during the rainy season.

It was found that under Puerto Rican conditions pink bollworms in the diapause always occur most abundantly during periods of

drought, regardless of the age of the crop or the time of the year at which the drought takes place. A few resting larvae always occur in the field regardless of the amount of rainfall—an indication of the existence of an inherited cycle which cannot be broken within several generations.

A total of 1,528 larvae were reared in the laboratory on food of a known moisture content. The mature larvae were classified as resting or nonresting individuals, and the water content of these two groups was determined.

The moisture content of the seed and lint was inversely proportional to the age of the boll, the coefficient of correlation being −0.986. The moisture content of the seed and lint was also inversely proportional to the percentage of resting larvae, the coefficient of correlation being −0.397.

The moisture content of resting larvae was found to be 7.9 percent lower than the moisture content of nonresting larvae.

Under tropical conditions any factor that limits the moisture content of the boll, the insect's food, tends to induce the development of the diapause.

A low relative humidity combined with a high temperature reduces the water content of the larvae by evaporation, thereby decreasing the rate of metabolism and causing arrested development.

Dry food caused by drought conditions, i. e., lack of rainfall, high temperatures, low humidity, excessive wind movement, high evaporation, and lack of available moisture for the plants, reduces the water content of the larvae themselves.

Moisture is the most important factor terminating the diapause under tropical conditions; and in the field, under conditions of heavy rainfall and high temperature, pupation and emergence of a majority of the resting larvae would be completed within 2½ to 3 months.

These studies suggest the possibility of a practical method for reducing pink bollworm damage, since the abundance of resting larvae in the field and, therefore, the carry-over, may be partly controlled by adjusting the planting date to the distribution of rainfall or by irrigation.

LITERATURE CITED

(1) Babcock, K. W.
1924. ENVIRONMENTAL STUDIES ON THE EUROPEAN CORN BORER (PYRAUSTA NUBILALIS HBN.). Jour. Econ. Ent. 17: 120-125, illus.

(2) ——
1927. THE EUROPEAN CORN BORER PYRAUSTA NUBILALIS HBN.: I. A DISCUSSION OF ITS DORMANT PERIOD. Ecology 8: 45-59.

(3) Ballard, E.
1921. RESULTS OF INVESTIGATION OF BIONOMICS OF PLATYEDRA GOSSYPIELLA, SAUNDERS, IN SOUTH INDIA, TOGETHER WITH SOME NOTES ON EARIAS INSULANA AND E. FABIA. Pusa Ent. Meeting Proc. 4 (1921): 70-83, illus.

(4) Ballou, H. A.
1920. THE PINK BOLL WORM (GELECHIA GOSSYPIELLA, SAUNDERS) IN EGYPT IN 1916-1917. Egypt Min. Agr. 120 pp., illus. Cairo.

(5) Baumberger, J. Percy.
1914. STUDIES IN THE LONGEVITY OF INSECTS. Ent. Soc. Amer. Ann. 7: 323-358, illus.

(6) BAUMBERGER, J. PERCY—Continued
1917. HIBERNATION: A PERIODICAL PHENOMENON. Ent Soc. Amer. Ann 10: 179–186, illus.
(7) BEDFORD, H. W.
1934. PROBLEMS CONNECTED WITH THE CONTROL OF THE PINK BOLLWORM, (PLATYEDRA GOSSYPIELLA, SAUNDERS) IN THE SUDAN. Empire Cotton Growing Corp., 2d Conf. Cotton Growing Prob., Proc., pp. 167–175.
(8) BISHARA, IBRAHIM.
1930. RATOON COTTON IN RELATION TO INSECT PESTS. Egypt Min. Agr. Tech. and Sci. Serv. Bul. 96, 68 pp., illus.
(9) BODENHEIMER, F. S.
1938. PROBLEMS OF ANIMAL ECOLOGY. 183 pp., illus. London.
(10) BODINE, JOSEPH HALL.
1921. FACTORS INFLUENCING THE WATER CONTENT AND THE RATE OF METABOLISM OF CERTAIN ORTHOPTERA. Jour. Expt. Zool. 32: 137-164, illus.
(11) ———
1923. HIBERNATION IN ORTHOPTERA. I. PHYSIOLOGICAL CHANGES DURING HIBERNATION IN CERTAIN ORTHOPTERA. Jour. Expt. Zool. 37: 457-476, illus.
(12) ———
1932. HIBERNATION AND DIAPAUSE IN CERTAIN ORTHOPTERA. II. RESPONSE TO TEMPERATURE DURING HIBERNATION AND DIAPAUSE. III. DIAPAUSE—A THEORY OF ITS MECHANISM. Physiol. Zool. 5: 538-548 and 549-554, illus.
(13) BOYCE, A. M.
1931. THE DIAPAUSE PHENOMENON, IN INSECTS, WITH SPECIAL REFERENCE TO RHAGOLETIS COMPLETA CRESS. (DIPTERA: TRYPETIDAE). Jour. Econ. Ent. 24: 1018-1024.
(14) BREDO, H.-J.
1934. LA LUTTE CONTRE LE VER ROSE (PECTINOPHORA GOSSYPIELLA SAUND.) PAR LA DÉSINFECTION DES GRAINES DE COTON AU MOYEN D'APPAREILS À AIR CHAUD. Belgium Min. des Colon., Dir. Gén. de l' Agr. Bul. Agr. du Congo Belge 25: [259]-270, illus.
(15) BREITENBECHER, J. K.
1918. THE RELATION OF WATER TO THE BEHAVIOR OF THE POTATO BEETLE IN A DESERT. *In* Tower, William Lawrence, The Mechanism of Evolution in Leptinotarsa. Carnegie Inst. Wash. Pub. 263, pp. 341-384, illus.
(16) BUXTON, PATRICK A.
1932. TERRESTRIAL INSECTS AND THE HUMIDITY OF THE ENVIRONMENT. Cambridge Phil. Soc. Rev. Biol. 7: [275]-320, illus.
(17) CHAPMAN, A. J., and CAVITT, H. S.
1934. THE INFLUENCE OF SOIL MOISTURE UPON SURVIVAL OF THE PINK BOLLWORM. Jour. Econ. Ent. 27: 820-827, illus.
(18) CHAPMAN, ROYAL N.
1931. ANIMAL ECOLOGY, WITH ESPECIAL REFERENCE TO INSECTS. 464 pp., illus. New York.
(19) COUSIN, GERMAINE.
1932. ÉTUDE EXPÉRIMENTALE DE LA DIAPAUSE DES INSECTES. Sup. Bul. Biol. de la France et Belg. 15, 341 pp., illus.
(20) DITMAN, L. P., WEILAND, G. S., and GUILL, J. H., JR.
1940. THE METABOLISM IN THE CORN EARWORM. III. WEIGHT, WATER, AND DIAPAUSE. Jour. Econ. Ent. 33: 282-295, illus.
(21) DOUGLASS, J. R.
1928. PRECIPITATION AS A FACTOR IN THE EMERGENCE OF EPILACHNA CORRUPTA FROM HIBERNATION. Jour. Econ. Ent. 21: 203-213, illus.
(22) ———
1933. ADDITIONAL INFORMATION ON PRECIPITATION AS A FACTOR IN THE EMERGENCE OF EPILACHNA CORRUPTA MULS. FROM HIBERNATION. Ecology 14: 286-297, illus.
(23) FENTON, F. A., and OWEN, W. L., JR.
1931. HIBERNATION OF PECTINOPHORA GOSSYPIELLA IN TEXAS. Jour. Econ. Ent. 24: 1197-1207, illus.

(24) FINK, DAVID E.
1925. PHYSIOLOGICAL STUDIES ON HIBERNATION IN THE POTATO BEETLE, LEPTINOTARSA DECEMLINEATA SAY. Biol. Bul. 49: 281-406, illus.
(25) GOUGH, LEWIS.
1916. THE LIFE HISTORY OF GELECHIA GOSSYPIELLA FROM THE TIME OF THE COTTON HARVEST TO THE TIME OF COTTON SOWING. Egypt Min. Agr. Tech. and Sci. Serv. Bul. 4, 16 pp.
(26) HODSON, A. C.
1937. SOME ASPECTS OF THE ROLE OF WATER IN INSECT HIBERNATION. Ecol. Monog. 7: 271-315, illus.
(27) ISLER, D. A., and FENTON, F. A.
1931. PRELIMINARY REPORT ON CONTROLLING PINK BOLLWORM IN TEXAS BY WINTER CULTURAL METHODS. Jour. Econ. Ent. 24: 795-807, illus.
(28) JOHNSTON, H. B.
1929. PINK BOLLWORM (PLATYEDRA GOSSYPIELLA, SAUNDERS) IN THE GEZIRA DISTRICT OF THE SUDAN IN 1927 AND 1928. Wellcome Trop. Res. Labs., Khartum, Ent. Sect. Bul. 26, 27 pp.
(29) KHAN, M. HAROON.
1938. STUDIES IN PLATYEDRA GOSSYPIELLA SAUNDERS (THE PINK BOLLWORM OF COTTON) IN THE PUNJAB. PT. IV, THE INCIDENCE OF PLATYEDRA GOSSYPIELLA IN RELATION TO CLIMATE (1926-1931). Indian Jour. Agr. Sci. 8: 191-214, illus.
(30) LOFTIN, U. C., MCKINNEY, K. B., and HANSON, W. K.
1921. REPORT ON INVESTIGATIONS OF THE PINK BOLLWORM OF COTTON IN MEXICO. U. S. Dept. Agr. Bul. 918, 64 pp., illus.
(31) PAYNE, NELLIE M.
1927. FREEZING AND SURVIVAL OF INSECTS AT LOW TEMPERATURES. Jour. Morph. and Physiol. 43: 521-546, illus.
(32) ———
1927. MEASURES OF INSECT COLD HARDINESS. Biol. Bul. 52: 449-457, illus.
(33) PICTET, ARNOLD.
1906. DES DIAPAUSES EMBRYONNAIRES, LARVAIRES ET NYMPHALES CHEZ LES INSECTES LÉPIDOPTÈRES. Genève Soc. Lépidoptérologique Bul. 1: 98-153, illus.
(34) ———
1907. DIAPAUSES HIBERNALES CHEZ LES LÉPIDOPTÈRES. Arch. Sci. Phys. Nat. 23: [302]-305.
(35) ———
1909. DIAPAUSES NYMPHALES CHEZ QUELQUES LÉPIDOPTÈRES. Arch. Sci. Phys. Nat. 27: 87-90.
(36) ———
1913. RECHERCHES EXPÉRIMENTALES SUR L'HIBERNATION DE LASIOCAMPA QUERCUS. Genève Soc. Lépidoptérologiques Bul. 2: [179]-206.
(37) ———
1913. NOUVELLES RECHERCHES SUR L'HIBERNATION DES LÉPIDOPTÈRES. Arch. Sci. Phys. Nat. 35: 301-304.
(38) RICE, PAUL L.
1937. EFFECT OF MOISTURE ON EMERGENCE OF THE RAGWEED BORER EPIBLEMA STRENUANA WALKER, AND ITS PARASITES. Jour. Econ. Ent. 30: 108-115, illus.
(39) ROBINSON, WILLIAM.
1928. WATER CONSERVATION IN INSECTS. Jour. Econ. Ent. 21: 897-902, illus.
(40) ———
1928. RESPONSE AND ADAPTATION OF INSECTS TO EXTERNAL STIMULI. Ent. Soc. Amer. Ann. 21: 407-417, illus.
(41) ROUBAUD, E.
1922. ÉTUDES SUR LE SOMMEIL D'HIVER PRÉ-IMAGINAL DES MUSCIDES. LES CYCLES D'ASTHÉNIE ET L'ATHERMOBIOSE RÉACTIVANTE SPÉCIFIQUE. Bul. Biol. de la France et Belg. 56: [455]-544, illus.
(42) SHELFORD, VICTOR E.
1929. LABORATORY AND FIELD ECOLOGY. 608 pp., illus. Baltimore.

(43) SPOONER, C. S.
1927. A STUDY OF THE CATALASE CONTENT OF CODLING MOTH LARVAE. Ill. Nat. Hist. Survey Bul. 16: 443-446.

(44) SQUIRE, F. A.
1937. A THEORY OF DIAPAUSE IN PLATYEDRA GOSSYPIELLA SAUND. Trop. Agr. (Trinidad) 14: 299-301, illus.

(45) ———
1940. OBSERVATIONS ON THE LARVAL DIAPAUSE OF THE PINK BOLLWORM, PLATYEDRA GOSSYPIELLA, SAUND. Bul. Ent. Res. 30: 475-481, illus.

(46) ———
1940. ON THE NATURE AND ORIGIN OF THE DIAPAUSE IN PLATYEDRA GOSSYPIELLA, SAUND. Bul. Ent. Res. 31: [1]-6, illus.

(47) STEINBERG, D. M., and KAMENSKY, S. A.
1936. LES PRÉMISSES OECOLOGIQUES DE LA DIAPAUSE DE LOXOSTEGE STICTICALIS L. (LEPIDOPTERA, PYRALIDAE). Bul. Biol. de la France et Belg. 70: [145]-183, illus.

(48) STOREY, G.
1921. THE PRESENT SITUATION WITH REGARD TO THE CONTROL OF THE PINK BOLLWORM IN EGYPT. Egypt Min. Agr. Tech. and Sci. Serv. Bul. 16, 16 pp.

(49) STRELNIKOV, I.
1936. WASSERUM SATZ UND DIAPAUSE BEI LOXOSTEGE STICTICALIS. Acad. des Sci. U. R. S. S. Compt. Rend. (Dok.) (n. s. 1) 10: [267]-271.

(50) TAYLOR, T. H. C.
1936. REPORT ON A YEAR'S INVESTIGATION OF PLATYEDRA GOSSYPIELLA (PINK BOLLWORM) IN UGANDA (MARCH, 1935, TO APRIL, 1936). Uganda Dept. Agr. Ann. Rpt. 1936 (2): 19-33.

(51) TOWNSEND, M. T.
1926. THE BREAKING-UP OF HIBERNATION IN THE CODLING MOTH LARVA. Ent. Soc. Amer. Ann. 19: 429-439.

(52) UVAROV, B. P.
1928. LOCUSTS AND GRASSHOPPERS, A HANDBOOK FOR THEIR STUDY AND CONTROL. 352 pp., illus. London.

(53) ———
1931. INSECTS AND CLIMATE. Ent. Soc. London Trans. 79: [1]-247, illus.

(54) WILLCOCKS, F. C.
1916. THE INSECT AND RELATED PESTS OF EGYPT, V. 1. THE INSECT AND RELATED PESTS INJURIOUS TO THE COTTON PLANT. PT. 1. THE PINK BOLLWORM. Sultanic Agr. Soc. 339 pp., illus. Cairo.

(55) WILLIAMS, C. B.
1924. THE PINK BOLLWORM IN EGYPT IN 1922. Egypt Min. Agr. Cotton Res. Bd. Ann. Rpt. 3 (1922): [1]-8, illus.

(56) WOLCOTT, G. N., and SEIN, F., Jr.
1931. LA ORUGA ROSADA DE LA CÁPSULA DEL ALGODÓN EN PUERTO RICO. Puerto Rico Insular Expt. Stn. Cir., 95, 13 pp., illus.

U. S. GOVERNMENT PRINTING OFFICE 1949

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington 25, D. C. - Price 10 cents

END